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ORIGINAL ARTICLE

Synthesis, characterization and antimicrobial activity of benzodioxane ring containing 1,3,4-oxadiazole derivatives

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KEYWORDS

1,4-Benzodioxane;
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Antibacterial;
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Abstract A series of 1,3,4-oxadizole derivatives containing 1,4-benzodioxane ring system were synthesized starting from 2,3-dihydro-1,4-benzodioxane-2-carbohydrazide. The synthesized compounds were characterized and evaluated for antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* and antifungal activity against *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans* by twofold serial dilution technique. Some of the synthesized compounds displayed comparable or even better antibacterial and antifungal activities than reference drugs norfloxacin, chloramphenicol and fluconazole, against tested strains.

1. Introduction

In recent years, the incidence of fungal and bacterial infections has increased dramatically. The widespread use of antifungal and antibacterial drugs resulted in resistance to drug therapy against fungal and bacterial infections which led to serious health hazards. The resistance of wide spectrum antifungal and antibacterial agents has initiated discovery and modification of the new antifungal and antibacterial drugs.

It is well-known that azole moieties are important pharmacophore that appear extensively in various types of pharmaceutical agents, widely implicated in biochemical processes and display diversity of pharmacological activities (Mamolo et al., 2005). A large number of azole compounds are used as antimicrobial drugs in clinic, for example, miconazole, clotrimazole and econazole are administered topically, while ketoconazole, itraconazole and fluconazole are useful in the treatment of systemic infections. Furthermore, it has been found that some azoles such as miconazole gave remarkable antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) (Guven et al., 2007). The

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widespread use of azole antimicrobial drugs led numerous efforts to develop some azole derivatives as new antimicrobial agents.

The compounds containing dioxane rings are of interest for the introduction of a variety of substituents into common skeleton, novel transformations, and can provide new and general routes to a variety of organic molecules. There are two important characteristics of these compounds, namely (i) readily opening to alkyletenes either under thermal or photochemical conditions and (ii) the C–C double bond, if present in the dioxane ring, acts as the enol form of masked acylacetic acids, which are important building blocks in organic syntheses. Benzodioxane represents a series of synthetic and natural compounds of considerable medicinal importance. Compounds containing dioxane ring systems exhibited different biological activities like antimicrobial (Mallesha and Mohana, 2011), antihepatotoxic (Ahmed et al., 2003; Khan et al., 2006), α-adrenergic blocking agent (Chapleo et al., 1983) and anti-inflammatory (Vazquez et al., 1997).

Oxadiazoles are an important type of oxygen and nitrogen containing aromatic heterocyclic compounds, possess desirable electronic and charge-transport properties and the various functional groups are easily introduced into the structurally rigid oxadiazole ring. These characteristics resulted in the extensive potential applications of oxadiazole based derivatives in the field of medicinal chemistry. Various methods have been reported recently for the synthesis of 1,3,4-oxadiazoles (Adib et al., 2009; Ramazani and Rezaei, 2010; Vechorkin et al., 2010). A large number of biological activities are associated with oxadiazole derivatives such as antitumor (Aboraia et al., 2006), anti-inflammatory (Palaska et al., 2002; Amir and Shikha, 2004), antimicrobial (Jha et al., 2010; Gilani et al., 2010; Manjunatha et al., 2010; El-Azab, 2007; Mamolo et al., 2005; Saleh et al., 2004), antifungal (Chen et al., 2008) and anticonvulsant (Zarghi et al., 2005).

In continuation to extend our research on antimicrobial compounds, we designed a series of new 1,3,4-oxadiazole derivatives containing 1,4-benzodioxane ring system. Herein, we wish to report the synthesis, antibacterial and antifungal activities of some novel 1,3,4-oxadiazole derivatives.

2. Experimental protocols

2.1. Chemistry

The IR spectra were recorded on Brucker. The mass spectra were recorded on a Bruker daltronics high resolution mass spectrometer, the ^{1}H NMR (300 MHz) was recorded on Bruker DPX 300 spectrometer in CD_3OD and DMSO-d_6 using TMS as internal standard reference and chemical shifts are in δ ppm. Elemental analyses were performed on Elementar Vario EL III, Carlo Erba 1108. The melting points were determined by capillary method.

2.1.1. Synthesis of ethyl-l,4-benzodioxane-2-carboxylate (*1*)

Anhydrous potassium carbonate (50 g) was added in portions to a stirred solution of 55 g of catechol in 200 mL of dry acetone followed by the dropwise addition of 34.5 g of ethyl-2,3-dibromopropionate. Another 50 g of potassium carbonate and 34.5 g of the dibromoester were added similarly and this was repeated two times more using a total of 200 g of potassium carbonate and 137.5 g of ester. Stirring and refluxing was continued for another 15 h. The reaction mixture was then filtered and the solid was washed several times with acetone. The filtrate was concentrated to about 75 mL and the residue was diluted with 50 mL of cold water. The oily layer was separated from the aqueous layer; the latter was extracted repeatedly with ether. The combined oily layer and ether extracts were washed with water, dried over magnesium sulfate, and evaporated. The dark residue was distilled at 96–97 °C (0.1 mm/Hg) to yield 38 g of ester **1** as a colorless semisolid. ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 1.23 (3H, t, J = 7.1 Hz, CH_3-12), 4.20 (2H, q, J = 7.1, 5.7 Hz, CH_2-12), 4.30 (2H, d, J = 2.7, CH_2-3), 4.77 (1H, t, J = 2.7, CH-2), 6.84 (4H, m, Ar-H); FTIR cm^{-1}: 3052 (=C–H, aromatic), 1772 (C=O), 1653 (C=C), 1292 (C–O, ester).

2.1.2. Synthesis of 2,3-dihydro-1,4-benzodioxane-2-carbohydrazide (*2*)

To a solution of ethyl-1,4-benzodioxane-2-carboxylate (0.01 mol) in ethanol (20 mL), hydrazine hydrate (0.01 mol) was added and the reaction mixture was refluxed. The progress of the reaction was monitored by TLC. After the completion of the reaction (usually 16 h), the excess solvent was removed under reduced pressure. The reaction mixture was poured over crushed ice. The solid thus separated was filtered, dried and crystallized with methanol to give a white powder; m.p.: 110–112 °C; Yield: 80%; ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 3.91 (2H, brs, NH_2-13), 4.24 (1H, dd, J = 6.0, 11.4 Hz, H_a-3), 4.46 (1H, dd, J = 6.0, 11.4 Hz, H_b-3), 4.78 (1H, d, J = 6.0, CH_2), 6.91 (4H, m, Ar-H), 7.78 (1H, s, NH-12); FTIR (KBr) cm^{-1}: 3052 (=C–H, aromatic), 1772 (C=O), 1673 (C=C), 1259 (–NH_2), 1195 (–NH), 758 (C=C); Anal Calcd. for $C_9H_{10}N_2O_3$ (%): C, 55.67; H, 5.19; N, 14.43; O, 24.72. Found: C, 55.37; H, 5.02; N, 14.67; O, 24.73.

2.1.3. Synthesis of 2-(phenyl)-5-(2,3-dihydro-1,4-benzodioxane-2-yl)-1,3,4-oxadiazole (*3a*)

A solution of 0.01 mol of 2,3-dihydro-1,4-benzodioxane-2-carbohydrazide, 0.01 mol benzoic acid and 5 mL of $POCl_3$ was refluxed with stirring for 6–7 h. The reaction mixture was cooled and poured over crushed ice. The precipitate thus obtained was filtered washed with sodium bicarbonate, dried and recrystallised with benzene: methanol. ^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 4.33 (2H, m, unresolved doublet, CH_2-3), 5.02 (1H, brs, unresolved doublet, CH-2), 6.88–7.67 (4H, m, Ar-H, ring A), 7.87 (5H, m, Ar-H, ring B); FTIR (KBr) cm^{-1}: 3162 (=C–H, aromatic), 1678 (C=C), 1492 (C=N), 1078 (C–O–C). HR-MS (m/z): 281.1970 [MH]$^+$ (Calcd. for $C_{16}H_{12}N_2O_3$, 280.2782); Anal Calcd. for $C_{16}H_{12}N_2O_3$ (%): C, 68.56; H, 4.32; N, 9.99; O, 17.13; Found: C, 68.46; H, 4.42; N, 10.05; O, 17.12.

2.1.4. 2-(2-Bromo-phenyl)-5-(2,3-dihydro-1,4-benzodioxane-2-yl)-1,3,4-oxadiazole (*3b*)

^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 4.24 (2H, m, unresolved doublet, CH_2-3), 5.15 (1H, brs, unresolved doublet, CH_2-2), 6.67–7.91 (4H, m, Ar-H, ring A), 7.65 (5H, m, Ar-H, ring B); FTIR (KBr) cm^{-1}: 3069 (=C–H, aromatic), 1670

(C═C), 1485 (C═N), 1067 (C–O–C), 756 (C-Br); Anal Calcd. for $C_{16}H_{11}BrN_2O_3$ (%): C, 53.50; H, 3.09; N, 7.80; O, 13.36; Found: C, 53.43; H, 3.19; N, 7.67; O, 13.43.

*2.1.5. 2-(3-Bromo-phenyl)-5-(2,3-dihydro-1,4-benzodioxane-2-yl)-1,3,4-oxadiazole (**3c**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 4.26 (2H, m, unresolved doublet, CH_2-3), 5.41 (1H, brs, unresolved doublet, CH_2-2), 6.58–7.23 (4H, m, Ar-H, ring A), 7.56 (5H, m, Ar-H, ring B); FTIR (KBr) cm^{-1}: 3106 (═C–H, aromatic), 1654 (C═C), 1498 (C═N), 1053 (C–O–C), 768 (C-Br); Anal Calcd. for $C_{16}H_{11}BrN_2O_3$ (%): C, 53.50; H, 3.09; N, 7.80; O, 13.36: Found: C, 53.45; H, 3.08; N, 7.84; O, 13.43.

*2.1.6. 2-(4-Bromo-phenyl)-5-(2,3-dihydro-1,4-benzodioxane-2-yl)-1,3,4-oxadiazole (**3d**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 4.35 (1H, dd, J = 5.4, 9.9 Hz, CH_2-3, H-α), 4.62 (1H, dd, J = 3.3, 3.2 Hz, CH_2-3, H-β), 5.97 (1H, brs, unresolved doublet CH-2), 6.87–7.19 (4H, m, Ar-H, ring A), 7.47–8.02 (4H, m, Ar-H, ring B); FTIR (KBr) cm^{-1}: 3156 (═C–H, aromatic), 1687 (C═C), 1493 (C═N), 1043 (C–O–C), 746 (C-Br); HRMS (m/z): 359.1955 $[M]^+$ (Calcd. for $C_{16}H_{11}BrN_2O_3$, 359.1742). Anal Calcd. for $C_{16}H_{11}BrN_2O_3$ (%): C; 53.50; H, 3.09; Br, 22.25; N, 7.80; O, 13.36. Found: C, 53.48; H, 3.15; N, 7.78; O, 13.26.

*2.1.7. 2-(2-Chloro-phenyl)–5-(2,3-dihydro-1,4-benzodioxane-2-yl)-1,3,4-oxadiazole (**3e**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 4.92 (2H, m (unresolved doublet), CH_2-3), 5.62 (1H, brs, unresolved doublet, CH-2), 6.74–7.82 (4H, m, Ar-H, ring A), 7.02–7.39 (4H, m, Ar-H, ring B); FTIR (KBr) cm^{-1}: 3197(═C–H, aromatic), 1648 (C═C), 1489 (C═N), 1028 (C–O–C), 745 (C-Cl). Anal Calcd. for $C_{16}H_{11}ClN_2O_3$ (%): C, 61.06; H, 3.52; N, 8.90; O, 15.25; Found: C, 61.12; H, 3.45; N, 8.87; O, 15.29.

*2.1.8. 2-(3-Chloro-phenyl)-5-(2,3-dihydro-1,4-benzodioxane-2-yl)-1,3,4-oxadiazole (**3f**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 4.54 (2H, m, unresolved doublet, CH-3), 5.22 (1H, brs, unresolved doublet, CH-2), 6.88–7.57 (4H, m, Ar-H, ring A), 7.23–7.45 (5H, m, Ar-H, ring B); FTIR (KBr) cm^{-1}: 3057 (═C–H, aromatic), 1643 (C═C), 1468 (C═N), 1023 (C–O–C), 768 (C-Cl). Anal Calcd. for $C_{16}H_{11}ClN_2O_3$ (%): C, 61.06; H, 3.52; Cl, 11.26; N, 8.90; O, 15.25; Found: C, 61.03; H, 3.48; N, 8.78: O, 15.30.

*2.1.9. 2-(4-Chloro-phenyl)-5-(2,3-dihydro-1,4-benzodioxane-2-yl)-1,3,4-oxadiazole (**3g**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 4.25 (2H, m, unresolved doublet, CH-3), 5.02 (1H, brs, unresolved doublet, CH_2-2), 6.88–7.67 (4H, m, Ar-H, ring A), 7.87 (5H, m, Ar-H, ring A); FTIR (KBr) cm^{-1}: 3158 (═C–H, aromatic), 1642 (C═C), 1475 (C═N), 1016 (C–O–C), 743 (C-Cl) Anal Calcd. for $C_{16}H_{11}ClN_2O_3$ (%): C, 61.06; H, 3.52; Cl, 11.26; N, 8.90; O, 15.25. Found: C, 60.98; H, 3.48; N, 8.85; O, 15.30.

*2.1.10. 2-(2,4-Dichloro-phenyl)-5-(2,3-dihydro-1,4-benzodioxane-2-yl)-1,3,4-oxadiazole (**3h**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 4.35 (2H, m, unresolved doublet, CH_2-3), 5.91 (1H, brs, unresolved doublet, CH-2), 6.88–7.07 (4H, m, Ar-H, ring A), 7.73–7.92 (3H, m, Ar-H, ring B); FTIR (KBr) cm^{-1}: 3050 (═C–H, aromatic), 1693 (C═C), 1478 (C═N), 1070 (C–O–C), 827, 734 (C-Cl). Anal Calcd. for $C_{16}H_{10}Cl_2N_2O_3$ (%): C, 55.04; H, 2.89; Cl, 20.31; N, 8.02; O, 13.75. Found: C, 54.94; H, 2.75; Cl, 20.28; N, 8.53; O, 13.65.

*2.1.11. 2-(2-Methyl-phenyl)-5-(2,3-dihydro-1,4benzodioxane-2-yl)-1,3,4-oxadiazole (**3i**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 2.35 (3H, s, Ar-CH_3), 4.52 (2H, m, unresolved doublet, CH_2-3), 5.17 (1H, brs, unresolved doublet, CH-2), 6.78–7.57 (4H, m, Ar-H, ring A), 7.12–7.46 (4H, m, Ar-H, ring B); FTIR (KBr) cm^{-1}: 3048 (═C–H, aromatic), 2970 (Ar-CH_3), 1638 (C═C), 1474 (C═N), 1025 (C–O–C); Anal Calcd. for $C_{17}H_{14}N_2O_3$ (%): C, 69.38; H, 4.79; N, 9.52; O, 16.31. Found: C, 69.25; H, 4.72; N, 9.54; O, 16.34.

*2.1.12. 2-(3-Methyl-phenyl)-5-(2,3-dihydro-1,4-benzodioxane-2-yl)-1,3,4-oxadiazole (**3j**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 2.42 (3H, s, Ar-CH_3), 4.41 (1H, dd, J = 5.4, 12.3 Hz, CH_2-3, H-α), 4.62 (1H, dd, J = 2.1, 7.9 Hz, CH_2-3, H-β), 5.18 (1H, brs, unresolved doublet CH-2), 6.88–7.01 (4H, m, Ar-H, ring A), 7.25–7.97 (4H, m, Ar-H, ring B); FTIR (KBr) cm^{-1}: 3197 (═C–H, aromatic), 2950 (Ar-CH_3), 1687 (C═C), 1490 (C═N), 1076 (C–O–C); Anal Calcd. for $C_{17}H_{14}N_2O_3$(%): C, 69.38; H, 4.79; N, 9.52; O, 16.31; Found: C, 69.46; H, 4.78; N, 9.49; O, 16.27.

*2.1.13. 2-(4-Methyl-phenyl)-5-(2,3-dihydro-1,4-benzodioxane-2-yl)-1,3,4-oxadiazole (**3k**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 2.26 (3H, s, Ar-CH_3), 4.27 (2H, m, unresolved doublet, CH_2-3), 5.43 (1H, brs, unresolved doublet, CH-2), 6.68–7.37 (4H, m, Ar-H, ring A), 7.34–7.87 (4H, m, Ar-H, ring B); FTIR (KBr) cm^{-1}: 3142 (═C–H, aromatic), 2850 (Ar-CH_3), 1668 (C═C), 1475 (C═N), 1038 (C–O–C); Anal Calcd. for $C_{17}H_{14}N_2O_3$ (%): C, 69.38; H, 4.79; N, 9.52; O, 16.31; Found: C, 69.42; H, 4.81; N, 9.48; O, 16.29.

*2.1.14. 2-(4-Hydroxy-phenyl)-5-(2,3-dihydro-1,4-benzodioxane-2-yl)-1,3,4-oxadiazole (**3l**)*

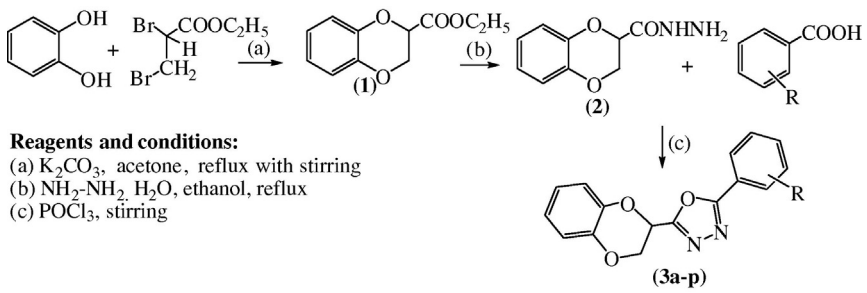
^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 4.37 (2H, m, unresolved doublet, CH_2-3), 5.26 (1H, brs, unresolved doublet, CH-2), 6.88–7.67 (4H, m, Ar-H, ring A), 7.26–7.34 (4H, m, Ar-H, ring B), 10.24 (1H, s, ArOH); FTIR (KBr) cm^{-1}: 3145 (═C–H, aromatic), 1646 (C═C), 1479 (C═N), 1023 (C–O–C); Anal Calcd. for $C_{16}H_{12}N_2O_4$ (%): C, 64.86; H, 4.08; N, 9.46; O, 21.60; Found: C, 64.82; H, 4.25; N, 9.45; O, 21.56.

*2.1.15. 2-(3,4-Dihydroxy-phenyl)-5-(2,3-dihydro-1,4-benzodioxane-2-yl)-1,3,4-oxadiazole (**3m**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 4.39 (2H, m (unresolved doublet), CH_2-3), 5.02 (1H, brs, unresolved doublet, CH-2), 6.88–7.05 (4H, m, Ar-H, ring A), 6.26–7.12(3H, m, Ar-H, ring B), 10.36 (2H, s, Ar-OH); FTIR (KBr) cm^{-1}: 3042 (═C–H, aromatic), 1648 (C═C), 1469 (C═N), 1048 (C–O–C); Anal Calcd. for $C_{16}H_{11}N_2O_5$ (%): C, 61.54; H, 3.87; N, 8.97; O, 25.62; Found: C, 61.58; H, 3.85; N, 8.89; O, 25.59.

*2.1.16. 2-(4-Methoxy-phenyl)-5-(2,3-dihydro-1,4-benzodioxane-2-yl)-1,3,4-oxadiazole (**3n**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 3.84 (3H, s, Ar-OCH_3), 4.62 (2H, m, unresolved doublet, CH_2-3), 5.87 (1H,



Scheme 1 Synthetic route for the preparation of 1,3,4-oxadiazole derivatives **(3a–p).**

brs, unresolved doublet, CH-2), 6.91–7.16 (4H, m, Ar-H, ring A), 7.92–7.94 (4H, m, Ar-H, ring B); FTIR (KBr) cm^{-1}: 3062 (=C–H, aromatic), 1611 (C=C), 1494 (C=N), 1180, 1017 (C–O–C); Anal Calcd. for $C_{17}H_{14}N_2O_4$ (%): C, 65.80; H, 4.55; N, 9.03; O, 20.62; Found: C, 65.78; H, 4.58; N, 9.13; O, 20.69.

*2.1.17. 2-(3,4-Dimethoxy-phenyl)-5-(2,3-dihydro-1,4-benzodioxane-2-yl)-1,3,4-oxadiazole (**3o**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 3.76 (2H, s, Ar-OCH_3), 4.52 (2H, m, unresolved doublet, CH_2-3), 5.35 (1H, brs, unresolved doublet, CH-2), 6.88–7.67 (4H, m, Ar-H, ring A), 7.01–7.32 (3H, m, Ar-H, ring B); FTIR (KBr) cm^{-1}: 3067 (=C–H, aromatic), 1664 (C=C), 1469 (C=N), 1245, 1030, 1024 (C–O–C); Anal Calcd. for $C_{18}H_{16}N_2O_5$ (%): C, 63.52; H, 4.74; N, 8.23; O, 23.51; Found: C, 63.48; H, 4.79; N, 8.26; O, 23.49.

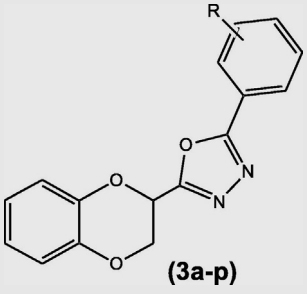
*2.1.18. 2-(4-Amino-phenyl)-5-(2,3-dihydro-1,4-benzodioxane-2-yl)-1,3,4-oxadiazole (**3p**)*

^{1}H NMR (300 MHz, DMSO-d_6): δ ppm 4.35 (2H, s, Ar-NH_2), 4.61 (2H, m (unresolved doublet), CH_2-3), 5.25 (1H, brs, unresolved doublet, CH-2), 6.73–7.21 (4H, m, Ar-H, ring A), 7.66–8.15 (4H, m, Ar-H, ring B); FTIR (KBr) cm^{-1}: 3072 (=C–H, aromatic), 1648 (C=C), 1449 (C=N), 1320 (C–N), 1036 (C–O–C); Anal Calcd. for $C_{16}H_{13}N_3O_3$ (%): C, 65.08; H, 4.44; N, 14.23; O, 16.25; Found: C, 65.10; H, 4.45; N, 14.24; O, 16.21.

2.2. Experimental determination of antibacterial and antifungal activities

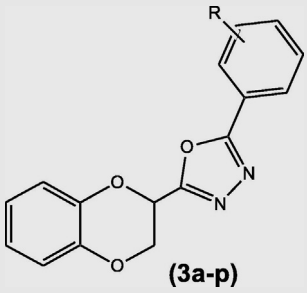
The minimal inhibitory concentrations (MIC_{50}) of the title compounds were determined *in vitro* by the modified micro-broth dilution method according to the methods defined by

Table 1 Chemical structures, melting point and percentage yield of the synthesized 1,3,4-oxadiazole derivatives **(3a–p)**.



S. No.	Compd.	R	Molecular formula	m.p. (°C)	Yield (%)
1	**3a**	H	$C_{16}H_{12}N_2O_3$	160–62	76
2	**3b**	2-Bromo	$C_{16}H_{11}BrN_2O_3$	175–77	68
3	**3c**	3-Bromo	$C_{16}H_{11}BrN_2O_3$	156–58	72
4	**3d**	4-Bromo	$C_{16}H_{11}BrN_2O_3$	135–37	75
5	**3e**	2-Chloro	$C_{16}H_{11}ClN_2O_3$	189–91	69
6	**3f**	3-Chloro	$C_{16}H_{11}ClN_2O_3$	201–203	82
7	**3g**	4-Chloro	$C_{16}H_{11}ClN_2O_3$	182–84	73
8	**3h**	2,4-Dichloro	$C_{16}H_{10}Cl_2N_2O_3$	148–150	79
9	**3i**	2-Methyl	$C_{17}H_{18}N_2O_4$	139–41	70
10	**3j**	3-Methyl	$C_{17}H_{14}N_2O_3$	134–36	81
11	**3k**	4-Methyl	$C_{17}H_{14}N_2O_3$	142–44	77
12	**3l**	4-Hydroxy	$C_{16}H_{12}N_2O_4$	128–30	64
13	**3m**	3,4-Dihydroxy	$C_{16}H_{11}N_2O_5$	165–67	65
14	**3n**	4-Methoxy	$C_{17}H_{14}N_2O_4$	87–89	71
15	**3o**	3,4-Dimethoxy	$C_{18}H_{16}N_2O_5$	141–43	74
16	**3p**	4-Amino	$C_{16}H_{13}N_3O_3$	55–57	67

Table 2 Antibacterial and antifungal data as MIC (μg/mL) for oxadiazole derivatives (**3a–p**).[a,b]



Compd.	R	Antibacterial activity			Antifungal activity		
		S. aureus	*E. coli*	*B. subtilis*	*A. niger*	*A. flavus*	*C. albicans*
3a	H	32	16	32	64	64	> 32
3b	2-Br	8	16	8	32	32	> 32
3c	3-Br	4	4	8	64	64	> 16
3d	4-Br	0.25	0.25	0.5	32	> 32	64
3e	2-Cl	4	4	16	16	> 16	> 16
3f	3-Cl	8	8	8	16	16	> 32
3g	4-Cl	0.5	> 0.25	0.5	32	32	> 32
3h	2,4-Cl	0.5	1	0.5	1	16	32
3i	2-CH_3	32	32	64	32	> 64	64
3j	3-CH_3	32	64	64	16	8	16
3k	4-CH_3	32	64	> 32	16	16	32
3l	4-OH	32	64	> 16	32	32	128
3m	3,4-OH	16	8	8	8	8	12
3n	4-OCH_3	8	16	16	32	16	32
3o	3,4-OCH_3	8	16	16	16	8	8
3p	4-NH_2	32	64	16	64	32	64
Norfloxacin		0.25	0.5	1	–	–	–
Chloramphenicol		8	8	16	–	–	–
Fluonazole		–	–	–	16	8	16

[a] Minimum inhibitory concentrations were determined by micro-broth dilution method.
[b] *S. aureus*, *Staphylococcus aureus* NCIM 2079; *B. subtilis*, *Bacillus subtilis* NCIM 2439; *E. coli*, *Escherichia coli* NCIM 5051; *A. niger*, *Aspergillus niger* ATCC 1034; *A. flavus*, *Aspergillus flavus* MTCC 2799; *C. albicans*, *Candida albicans*, ATCC 753.

the National Committee for Clinical Laboratory Standards. The test strains were provided by the National chemical Laboratory, Pune. The prepared compounds were evaluated for their antibacterial activity against *S. aureus* NCIM 2079 and *Bacillus subtilis* NCIM 2439 as Gram-positive, *Escherichia coli* NCIM 5051 as Gram-negative bacteria. The bacterial suspension was adjusted with sterile saline to a concentration of 1×10^5 CFU. The test compounds were dissolved in dimethyl sulfoxide (DMSO) to prepare the stock solutions. The test compounds and reference drugs were prepared by twofold serial dilution to obtain the required concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5 and 0.25 μg/mL. These dilutions were inoculated and incubated at 37 °C for 24 h. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO at the same dilutions as used in the experiment. The new compounds were evaluated for their antifungal activity against *Aspergillus niger* ATCC 1034, *A. flavus* MTCC 2799 and *Candida albicans* ATCC 753. A spore suspension in sterile distilled water was prepared from 1-day old culture of the fungi growing on Sabouraud agar (SA) media. The final spore concentration was $1–5 \times 10^3$ spore mL^{-1}. From the stock solutions of the tested compounds and reference antifungal fluconazole, dilutions in sterile RPMI 1640 medium were made resulting in concentrations (0.25–512 μg/mL) of each tested compound. These dilutions were inoculated and incubated at 35 °C for 24 h. The drug MIC_{50} was defined as the first well with an approximate 50% reduction in growth compared to the growth of the drug-free well.

3. Result and discussion

The synthetic route used to prepare starting materials and the title compounds is outlined in Scheme 1. The starting material ethyl-l,4-benzodioxane-2-carboxylate **(1)** was prepared by reaction between catechol and ethyl-2,3-dibromopropionate in dry acetone in the presence of anhydrous potassium carbonate, which on treatment with hydrazine hydrate afforded the corresponding hydrazide **(2)**. The reaction of hydrazide **(2)** with substituted aryl carboxylic acids in phosphorus oxychloride ($POCl_3$) gave the cyclized products 2-(substituted-phenyl)-5-(2,3-dihydro-1,4-benzodioxane-2-yl)-1,3,4-oxadiazoles **(3a–p)**. Chemical structures, melting point and percentage yield of the synthesized compounds were reported in Table 1. The synthesized compounds were characterized by ^{1}H NMR, Mass and IR spectroscopical data and elemental analysis.

The IR spectrum of compound **1** showed an intense peak at 1772 cm^{-1} for carbonyl C═O; 1653 cm^{-1} for C═C; 3052 cm^{-1} for ═C–H; and 1292 cm^{-1} for C–O ester groups. The ^{1}H NMR spectrum of **1** showed a triplet at δ 1.23 (J = 7.1 Hz) and a quar-

tet at δ 4.20 (J = 7.1, 5.7 Hz) due to –CH_3 at position 12 and CH_2– at position 13, respectively. A triplet at δ 4.77 and a doublet at δ 4.3 were assigned to the protons of CH_2– at positions 2 and 3. Aromatic protons appeared as multiplets at δ 6.84–7.22. The IR spectrum of compound **2** showed an intense peak at 1725 cm^{-1} for carbonyl C=O, 1642 cm^{-1} for C=C, 3045 cm^{-1} for =C–H aromatic ring. The ^{1}H NMR spectrum of **2** showed two double doublets at δ 4.24 and 4.46 (J = 6.0) corresponding to C-H_a and C-H_b of position 2, a doublet at δ 4.78 was assigned to the protons of CH_2 at position 3, Two protons of NH_2– at position 13 were appeared as a broad singlet at δ 3.91 and a proton as a singlet at δ 7.78 (J = 7.5) was assigned to the protons of NH– at position 12. Aromatic protons were appeared as multiplets at δ 6.91 ppm. The compound **3a** showed peaks at 3162 for =C–H, aromatic ring), 1678 for C=C, 1492 for C=N and 1078 for C–O–C. The ^{1}H NMR spectrum of **3a** showed two protons of CH_2 at positions 2 and 3 as multiplets (unresolved doublet) at δ 4.33 and 5.02, respectively. Aromatic protons of ring A were appeared as multiplets at δ 6.88–7.67. Aromatic protons of ring B were located at δ 7.87. The mass spectrum of the title compounds is in conformity with the assigned structures. The mass spectra of these compounds showed molecular ion peaks corresponding to their molecular formulae.

All of the synthesized compounds were screened *in vitro* for antibacterial activities against Gram-positive *S. aureus* and *B. subtilis* and Gram-negative *E. coli* as well as antifungal activities against *A. niger*, *A. flavus and C. albicans* by twofold serial dilution technique (Kadi et al., 2007 Ozbek et al., 2007). All compounds were evaluated at the concentrations of the antimicrobial agents ranging from 0.25 to 512 μg/mL and scored for MIC_{50} as the level of growth inhibition of the microorganisms compared with that of the current antimicrobial drugs fluconazole, chloramphenicol and norfloxacin in clinic. The data of antibacterial and antifungal tests are depicted in Table 2.

The obtained results showed that the synthesized compounds **3a–p** exhibited moderate to excellent activities against all tested strains. As noted in Table 2, compound **3d, 3g, 3h** have shown excellent antibacterial activities against both the Gram-positive strains *S. aureus* and *B. subtilis* and Gram negative *E. coli* with MIC values of 0.25–1 μg/mL. Antimicrobial activity data revealed that the presence of electron withdrawing group in aromatic ring of 1,3,4-oxadiazole ring improved the activity; however, a more lipophilic group at the same position greatly enhanced the antifungal activities of the synthesized azole derivatives.

4. Conclusion

In conclusion, a series of 1,4-benzodioxane-based azole derivatives were designed and synthesized for the first time via an easy, convenient and efficient synthetic route. The antimicrobial results showed that azole in combination with 1,4-benzodioxane is a promising template for antibacterial and antifungal activities.

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